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PRINCIPAL INVESTIGATOR: Bianca P. Gomez  
Robert Clarke, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University  
Washington, DC 20007

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| <b>13. ABSTRACT (Maximum 200 Words)</b><br><p>The over-expression of the human X-base binding protein (h-XBP-1) and hence elevated levels of CRE transcription activator contributes to the antiestrogen resistance. Preliminary data using Western blot analysis indicates that E2-independent but responsive breast cancer cells that are responsive to antiestrogen showed elevated hXBP-1 protein level whereas, the cell line that is resistant to antiestrogen showed a lower level of hXBP-1 protein expression. Furthermore, a promoter-reporter assay measuring luciferase activity as an indicator of CRE activation showed that cells that are antiestrogen resistant had a four-fold increase in CRE activation when compared to the sensitive cells. HXBP-1 is transcription factor that belongs to the ATF/CRE family of transcription factors and can bind to and activate the CREs (cyclic AMP response elements). It is based on this relationship that the amount of hXBP-1 protein levels could be determined by measuring the level of CRE containing genes. In our experiments by over expressing hXBP-1 using transfection we well convert sensitive cells to resistant cell. On the other hand, cell resistant to antiestrogen will become sensitive by using an antisense oligonucleotide approach. Anchorage dependent as well as anchorage independent growth responses of the respective transformed cells to antiestrogen will also be measured.</p> |   |  |  |                                  |
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FOREWORD

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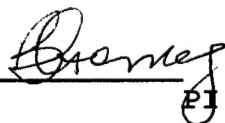
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N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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07-02-03



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Date

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## INTRODUCTION

Human X box bonding protein (hXBP-1) has been associated with antiestrogen resistance in our lab. We believe that by the up regulation of hXBP-1 due to the over-expression of this gene, that there is some contribution to antiestrogen resistance. This resistance to antiestrogens may occur by growth induction or by apoptosis blockage. Once we have up regulated hXBP-1 it is expected that there must be increased CRE transcriptional activation, this will be measured by promoter- reporter assays. The purpose of these experiments is based on the fact that most patients with initial responsive breast tumors to antiestrogens acquire antiestrogen resistance (1). Although several possible mechanisms such as mutations in the estrogen receptors (ER) and loss of the ER expression have been associated with antiestrogen resistance other possible mechanisms that can be considered to contribute to antiestrogen resistance may also include Tamoxifen stimulated resistance mechanisms (1). Tamoxifen (TAM) or ICI 46, 477 is the most widely used antiestrogen for breast cancer treatment. Faslodex (ICI 182, 780) is the other antiestrogen therapy used for breast cancer treatment (2). It is effective in patients who have acquired resistance to TAM treatment. In our experiments the two cell lines MCF7 and T 47D are being used to determine resistance to these antiestrogens when hXBP-1 is overexpressed.

## BODY

1. MCF7 cells sensitive to antistrogen were transfected with a pcDNA 3.1 vector (Invitrogen) containing a hXBP-1 insert for hXBP-1 over-expression. The empty vector was also transfected into MCF-7 cells to create a negative control.
2. Clone resistant to G 418 were selected to determine clones positive for the pcDNA 3.1/ hXBP-1 insert. Clones were expanded using 6 well plates.
3. hXBP-1 protein was extracted from these clones using Trizol reagent (Invitrogen). The amounts of proteins expressed in these cells were determined by Western blot analysis.
4. HXBP-1 activity was determined by measuring CRE transcriptional activation using a dual luciferase promoter- reporter assay system (Promega).

XBP-1  
M.W =28kDa

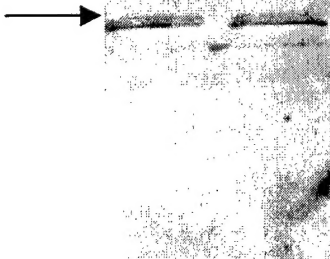


Fig. 1. Western blot to show two clones harboring hxbp-1 insert.

### Transcriptional Activation of CRE in MCF-7/hXBP-1 cells

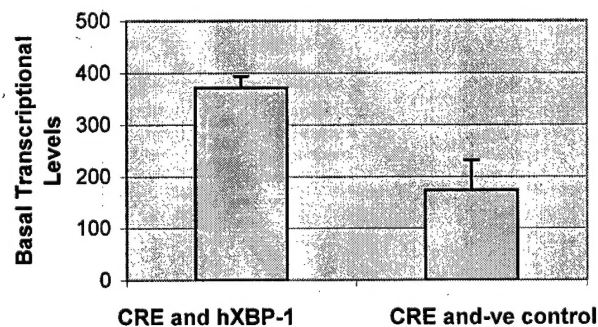


Fig.2. Transcriptional activation of hXBP-1 and CRE in MCF-7 cells. Negative control is empty pcDNA3.1 vector

#### KEY RESEARCH ACCOMPLISHMENTS

Task 1. To determine if sensitive cells can become resistant to antiestrogens Tamoxifen and Faslodex by the over-expression of hXBP-1 in breast cancer cells (Months 1-12):

- Transfected hXBP-1 into MCF/LCC1 (sensitive cells) with the vector containing the hXBP-1 insert.
- Cells were plated in media containing G418 to select resistant colonies (Months 1-6).
- Measured hXBP-1 expression levels in selected clones (Months 6-10).
- Confirmed hXBP-1 activity by measuring CRE transcriptional activity (Months 10-12).
- Currently T47D cells transfected with the pCDNA3.1 vector containing the hXBP-1 insert as well as the empty vector used as the negative control are now been grown in selective media for the selection of positive clones.

Course taken during the academic year 2001-2002: TBIO/PBIO  
595 Tumor Endocrinology (3 credits)

REPORTABLE OUTCOMES

None at this current time.



## CONCLUSION

- Over-expression of hXBP-1 was accomplished by the transfection of MCF-7 cells.
- Elevated levels of CRE in MCF7 cells transfected with the hXBP-1 indicate up regulation of hXBP-1.
- The MCF-7/hXBP-1 cells are now available for further characterization to explain the effects of XBP-1 expression on hormone responsiveness.

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